The semaphorin/collapsin family of proteins has been recognized as one important negative regulator of axon outgrowth and terminal arborization (Luo, *et al.*, 1993; Kolodkin, *et al.*, 1992, 1993). Chick collapsin-1 induces growth cone collapse and a cessation of neurite outgrowth from at least a subset of dorsal root ganglion neurons (Raper and Kapfhammer, 1990; Luo, *et al.*, 1993; hereinafter abbreviated "DRG"). Insect semaphorins have a demonstrated *in vivo* role during axonal pathfinding and synaptic terminal branching (Kolodkin, *et al.*, 1992; Matthes, *et al.*, 1995). There are at least 7 vertebrate semaphorins identified and there may be as many as 20 members of this family (Puschel, *et al.*, 1995; Messersmith, *et al.*, 1995; Luo, *et al.*, 1995; Inagaki, *et al.*, 1995; Adams, *et al.*, 1996). A decrease in actin filaments after collapsin-1 application has been documented (Fan, *et al.*, 1993). The mechanisms whereby collapsin-1 binding to an unidentified transmembrane receptor triggers this depolymerization is unclear.

B1

Delete the paragraph set out on page 4 at lines 1 to 9 and insert the following paragraph in its place:

In non-neuronal cells, the rho subfamily of monomeric ras-related GTP-binding proteins have prominent effects on the actin-based cytoskeleton and on cell shape (Hall, 1990; 1994). In fibroblasts, rho activation has been linked to stress fiber form ation and focal adhesions, rac1 activation with membrane ruffling and lamelipodia, and cdc42 activation with filopodial formation (Nobes and Hall, 1995). Single amino acid substitutions have been identified which produce constitutively active or dominant negative forms of each of these proteins. The C3 transferase from *Clostridium botulinum* (hereinafter abbreviated *C. botulinum*) ADP-ribosylates rho specifically and inactivates the G protein.

B2

Delete the paragraph set out on page 15 at lines 5 to 16 and insert the following paragraph in its place:

Comparison of collapsin-1 action with LPA and thrombin action. As a first step to assessing the role of small G proteins in collapsin action, the effect of readily available pharmacolgical agents on collapsin-1 action was compared to their effects on LPA and

B3

thrombin action. The myosin light chain kinase inhibitor, KT5926, blocks LPA-induced neurite retraction and also decreases the potency of recombinant collapsin-1 as a growth cone collapse factor (Figure 1A). A number of other agents had little or no effect on collapsin-1 action including tyrosine kinase inhibitors, protein kinase A inhibitors, voltage-sensitive Ca channel blockers and depolarization with KCl. The more general protein kinase inhibitor, staurosporine, and the protein kinase C activator, tissue plasminogen activator (hereinafter abbreviated "TPA"), both induced growth cone collapse at concentrations below 10 nM, but their action was not synergistic with collapsin-1.

Delete the paragraph set out on page 20 at line 20 to page 21, line 4 and insert the following paragraph in its place:

Mechanism of rac1 activation: dbl proteins, G protein cascade, Collapsin Response Mediator Protein (CRMP). The mechanism by which rac1 might be activated by extracellular collapsin-1 is unclear. In other cells types, proteins with domains homologous to the human Dbl act upstream of rac1 as guanine nucleotide exchange factors (Boguski and McCormick, 1993), but their presence in neuronal growth cones has not been studied. Receptors of several classes appear to be capable of activating rac1 in other cells, including receptor tyrosine kinases, serpentine receptors coupled to heterotrimeric G proteins and cytokine receptors of the TNF class. A central role for heterotrimeric G proteins in growth cone signal transduction is supported by a number of studies (Strittmatter, *et al.*, 1990; 1993; 1994b; 1995). Data presented here indicate that heterotrimeric G proteins (Figure 1B) may be involved in collapsin signaling. An intracellular family of neuronal proteins, CRMPs, has been identified; these are required for collapsin action but their interaction with other members of this signaling pathway is not established (Goshima, *et al.*, 1995; Wang and Strittmatter, 1996). There are no data indicating that intracellular calcium ion levels are likely to mediate collapsin action.

Please insert the attached Abstract as page 31.